

CHROMOSOME NUMBER AND MORPHOLOGY IN THE CONIFERS

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With plates 75-79 and three text figures

THE EXISTING CONIFERS are undoubtedly members of a very old group of plants. Although they constitute the predominant flora of the temperate zone, the number of genera is less than fifty, and many of these are of restricted distribution, and some are monotypic. As a whole, the genera are well differentiated on the basis of floral, anatomical, and gametophytic characters. Species characters are usually well marked and are frequently associated with geographic distribution. The stability of the Conifers would seem to indicate that evolution in this group of plants has passed its climax and that the existing forms are survivors of long natural selection.

A study of chromosome morphology in the Conifers should be of interest in relation to the distinct and long established differentiation of different genera. Previous cytological work shows that the basic chromosome number is usually 12 in the Conifers, and in all other Gymnosperms, with the exception of the Gnetales. Many of these counts were made incidental to morphological studies. A critical study of chromosome morphology has been made in only a few species.

The taxonomic grouping of the Conifers followed in this paper is based on Rehder's (1927) Manual. The Conifers are taken to include the Taxaceae and the Pinaceae. The Pinaceae are divided into four sub-families, the Cupressineae, Taxodineae, Araucarineae, and Abietineae. Representatives of all groups have been examined, with the exception of the Araucarineae. The analysis of the somatic chromosomes was done by the senior author, and the meiotic chromosomes were studied by the junior author.

MORPHOLOGY OF SOMATIC CHROMOSOMES

Preparations of root tips of Conifers are not suitable for a study of somatic chromosomes. The chromosomes are long, and the arms are usually oriented more or less at right angles to the metaphase plate so that polar views of division figures offer little opportunity for a comparison of individual chromosomes, and the numbers are too large to

permit an adequate study from side views of division figures. The somatic chromosomes are best observed in the early development of the endosperm. The chromosomes in this tissue are haploid in number, divisions are numerous, and the chromosomes can be studied from either polar or side views when flattened in aceto-carmine smears.

The entire endosperms were dissected out of the female cones and fixed in absolute alcohol-acetic acid, 70 parts of alcohol to 30 parts acetic. Aceto-carmine smears were made after the material had been fixed for several hours or longer. In some cases the endosperms were changed to 80 percent alcohol after fixing for 24 hours in the alcohol-acetic solution. Aceto-carmine smears can be obtained from such material at any time.

The endosperms were smeared in aceto-carmine containing enough haematoxylin and iron alum to produce a sharp stain. After the slide had been covered, the preparation was heated, and the cover pressed firmly with filter paper to flatten the cells and remove excess aceto-carmine. When sealed, such preparations remain in good condition for several weeks.

The various stages of mitosis in the endosperm of *Cephalotaxus* are shown in plate 75 in order to illustrate the types of figures obtained. The photographs are from preparations which had been flattened sufficiently to include all chromosomes of a given cell in approximately the same focus. During the prophase stage, a single large nucleolus is always found (Fig. 1). At metaphase the chromosomes are U-shaped, with the spindle fiber attachment points in approximately the same plane (Fig. 2). The chromosomes begin to separate at the fiber attachment point, and usually the opening at this region, or the protrusion of attachment points, can be observed at metaphase. The actual separation of daughter chromosomes proceeds rapidly, although occasional figures are found where the chromosomes are still associated at the distal ends (Fig. 3). At late anaphase the individual chromosomes are clearly defined, and the relative lengths of the arms are easily determined (Fig. 4). In *Cephalotaxus Fortuni* 11 chromosomes are approximately isobrachial and one is heterobrachial with a trabant at the end of the short arm. At telophase the chromosomes form a compact mass of chromatin (Fig. 5), and as the daughter nuclei are reorganized before passing into the resting stage, the chromosomes appear to be polarized and to surround a single large nucleolus (Fig. 6). The chromosomes vary considerably in size, even in the same endosperm. They seem to be larger in the free nuclear divisions and become

smaller when cell walls are formed. *Cephalotaxus* seems to have larger chromosomes than the other Conifers studied, but little or no consistent difference is found in the size of chromosomes of other genera.

The chromosomes of *Ginkgo biloba* were included in this study because this species is the only surviving member of the Ginkgoaceae. The haploid chromosome complement from endosperm tissue is represented in figure 7 (Pl. 76). Two of the 12 chromosomes are approximately isobrachial, while 10 of them have sub-terminal fiber attachment points.

The only available genera for study in Taxaceae were *Cephalotaxus* and *Taxus*. *Torreya* is represented in the Arnold Arboretum, but female cones are seldom produced. Twelve chromosomes were found in each of the three species of *Taxus* examined, *T. canadensis*, *T. baccata*, and *T. cuspidata* (Pl. 76, figs. 8, 9, and 10). In each case one chromosome has a terminal or subterminal attachment point, one is distinctly heterobrachial, while the others are more or less isobrachial.

In the Pinaceae, the sub-family Abietineae has been studied most extensively. The chromosomes of *Pinus* are especially clearly defined. Twelve chromosomes have been found in *P. Banksiana*, *P. Bungeana*, *P. flexilis*, *P. Jeffreyi*, *P. mugo rotundata*, *P. nigra*, *P. parviflora*, *P. peuce*, *P. ponderosa*, *P. resinosa*, *P. Strobus*, *P. sylvestris*, *P. Thunbergiana*, and *P. virginiana*. The chromosomes of all species of *Pinus* seem to be very similar. One of the 12 chromosomes is somewhat heterobrachial, and the others have approximately median fiber attachments. The chromosomes of three species, *P. parviflora*, *P. Thunbergiana*, and *P. ponderosa*, are illustrated (Pl. 76, figs. 11, 12 and 13).

The chromosomes of *Cedrus libanotica* are morphologically much like those of *Pinus* (Pl. 76, fig. 14). One of the 12 chromosomes is distinctly heterobrachial, and the others are approximately isobrachial.

Two species of *Larix* have been examined for chromosome morphology. The chromosomes of *L. Kaempferi* are shown at late anaphase in figure 15 (Pl. 76), and those of *L. decidua* at metaphase are shown in figure 16 (Pl. 76). The chromosomes of these two species are very similar; 6 of the 12 chromosomes are distinctly heterobrachial, and 6 have approximately median fiber attachment points.

The chromosomes of *Pseudolarix* are unusual among the Conifers, both in number and morphology. There are 22 chromosomes, 20 with terminal or subterminal fiber attachments, and two with more nearly median attachments, although both are distinctly heterobrachial (Pl.

76, fig. 17). The same condition was found in three different trees of *Pseudolarix amabilis*.

Twelve chromosomes have been found in all species of *Picea* examined. The chromosomes of *P. pungens* and *P. Abies* are very similar (Pl. 76, fig. 18 and Pl. 77, fig. 19). Three of the chromosomes are clearly heterobrachial, while the others are more or less isobrachial.

The chromosomes of *Tsuga canadensis* (Pl. 77, fig. 20) and *T. caroliniana* are very similar and resemble those of *Picea*. Three of the 12 chromosomes are distinctly heterobrachial, and 9 have approximately median fiber constrictions. One of the heterobrachial chromosomes has a secondary constriction.

Pseudotsuga taxifolia has 13 chromosomes. One of the chromosomes seems to have a completely terminal fiber attachment point, 6 are heterobrachial, and 6 have approximately median fiber constrictions. The fiber attachment points are not shown clearly in the drawing (Pl. 77, fig. 21), but in other figures the shortest chromosome opens out at one end and appears to have a completely terminal attachment point.

The chromosomes of *Abies cephalonica* and *A. concolor* appear to be similar (Pl. 77, figs. 22 and 23). Five of the 12 chromosomes are clearly heterobrachial, while the others are approximately isobrachial.

In the sub-family Taxodineae the endosperm chromosomes were examined in only one genus. *Cryptomeria japonica* has 11 chromosomes in the endosperm cells. Counts were made both at metaphase and late anaphase (Pl. 77, fig. 24). All chromosomes are approximately isobrachial. Root tip counts from aceto-carmine preparations gave a diploid chromosome number of 22 in *Taxodium distichum* and in *Taiwania cryptomerioides*, but absolutely accurate counts are difficult in root tip cells, even when the cells are flattened after mounting in aceto-carmine.

In the sub-family Cupressineae three typical genera were studied. There are 11 chromosomes in *Thuja occidentalis*, in *T. orientalis*, and in *T. plicata* (Pl. 77, figs. 25, 26 and 27). One or two chromosomes are somewhat heterobrachial, and the others have approximately median fiber attachments.

Both *Juniperus virginiana* and *J. rigida* have 11 chromosomes, most of which are more or less isobrachial (Pl. 77, figs. 28 and 29). In *J. rigida* one chromosome has a small trabant. *Chamaecyparis Lawsoniana* also has 11 chromosomes in the endosperm cells (Pl. 77, fig. 30). The chromosomes resemble those of *Thuja* and *Juniperus*. In all

genera of the Cupressineae examined a few chromosomes are somewhat heterobrachial and the others approximately isobrachial.

In order to facilitate a comparison of the chromosomes in the different families and genera of Conifers, diagrams of the chromosome complements have been made for the different genera studied. The relative lengths of each chromosome are indicated by vertical lines. The relative lengths of the arms of each chromosome are shown by placing the spindle fiber attachment point on the horizontal line. The chromosomes of each genus are placed in order, according to the length of the shorter arms. These diagrams are necessarily only approximately correct, and differences between species, and in some cases differences between genera, are of doubtful significance. The graphs are reliable in indicating the more conspicuous differences in chromosome morphology. These figures are shown in diagrams on text figures 1, 2 and 3.

A survey of these figures shows that the chromosomes of *Ginkgo* are distinctly different from those of any of the Conifers. The two representatives of the Taxaceae, *Cephalotaxus*, and *Taxus* are somewhat similar in chromosome morphology, although the chromosomes of *Cephalotaxus* appear to be larger, and the short heterobrachial chromosome has a secondary constriction on the short arm. The short heterobrachial chromosome of *Taxus* has a nearly terminal fiber attachment point.

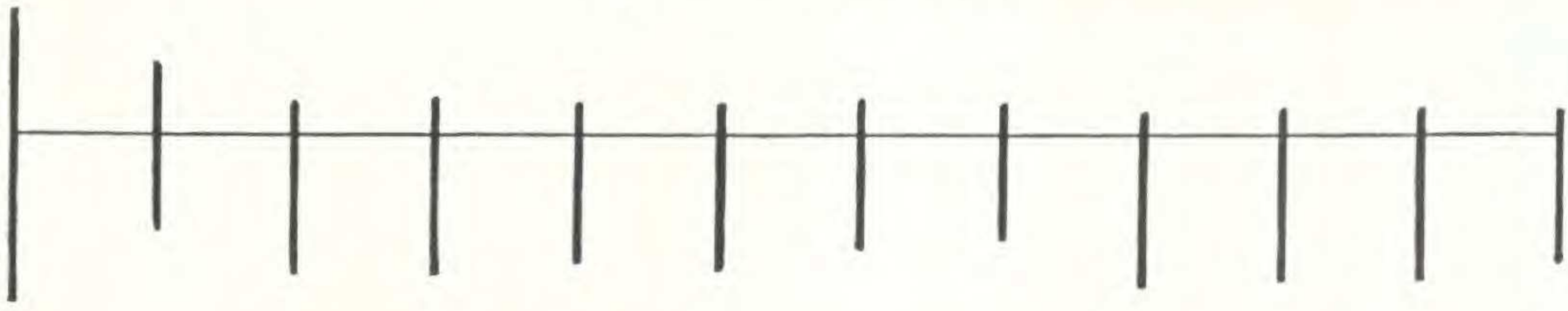
In the Pinaceae there is considerable variation in chromosome morphology between certain genera, even in the same sub-family. Little or no variation is found between diploid species of the same genus. All chromosomes of *Pinus* are approximately isobrachial. *Cedrus* differs from *Pinus* in possessing two slightly more heterobrachial chromosomes. Six of the chromosomes of *Larix* are heterobrachial. *Pseudotsuga* has 7 heterobrachial, and 5 isobrachial chromosomes, and one with a terminal fiber. *Picea* and *Tsuga* have similar chromosome morphology. *Pseudolarix* is unique among the Abietineae in having 22 chromosomes, only two of which are isobrachial. Five of the 12 chromosomes of *Abies* are distinctly isobrachial.

The chromosomes of the representatives of the Taxodineae and Cupressineae, i. e. *Cryptomeria*, *Juniperus*, *Chamaecyparis*, and *Thuja*, are similar in morphological characters, and the basic number is 11 for each genus.

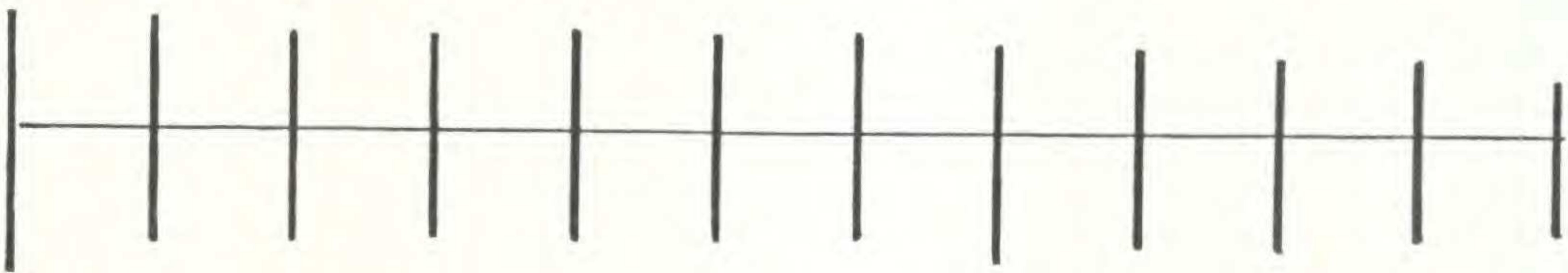
MEIOTIC DIVISIONS IN CONIFERS

A study of the meiotic divisions in different genera of Conifers was undertaken to determine chromosome numbers and the frequency and

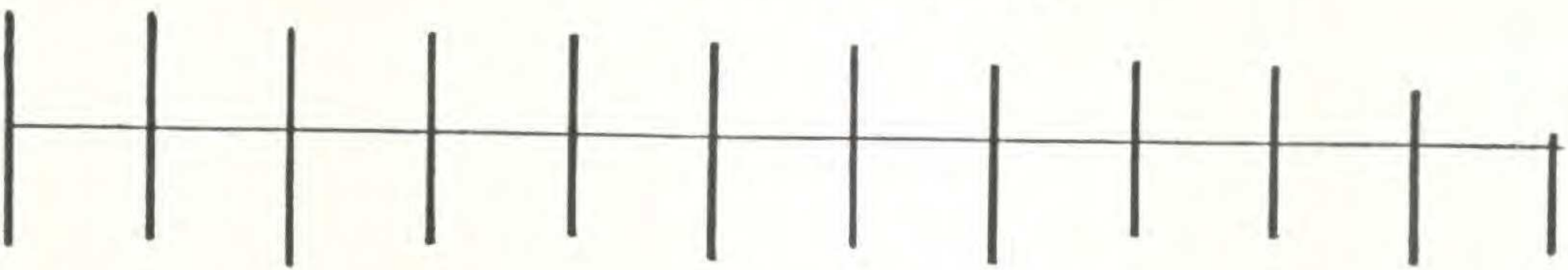
Ginkgo biloba



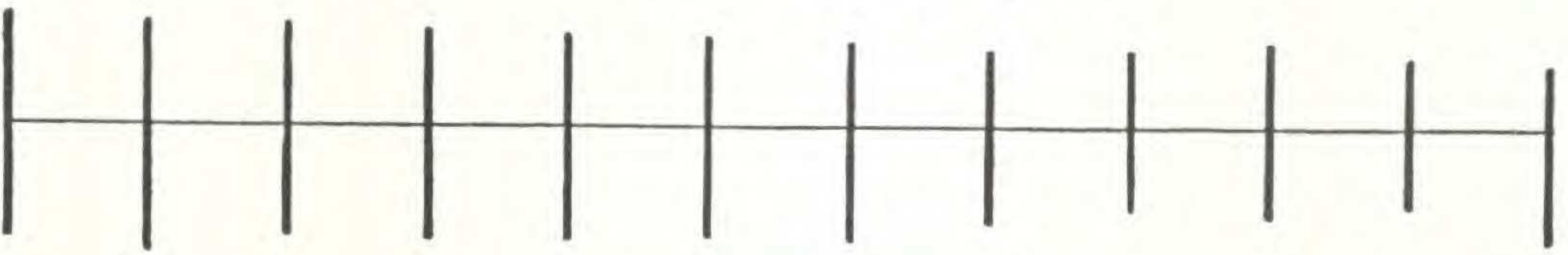
Cephalotaxus Fortunei



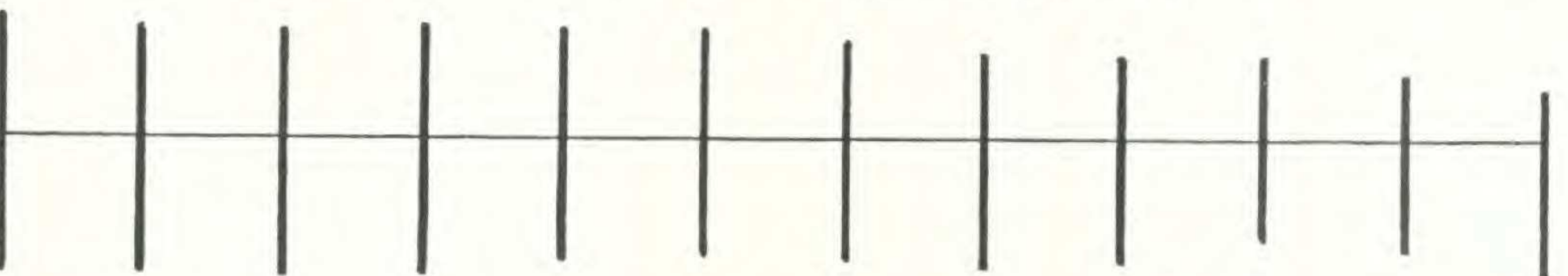
Taxus baccata



Pinus parviflora



Cedrus Libanotica



Larix decidua

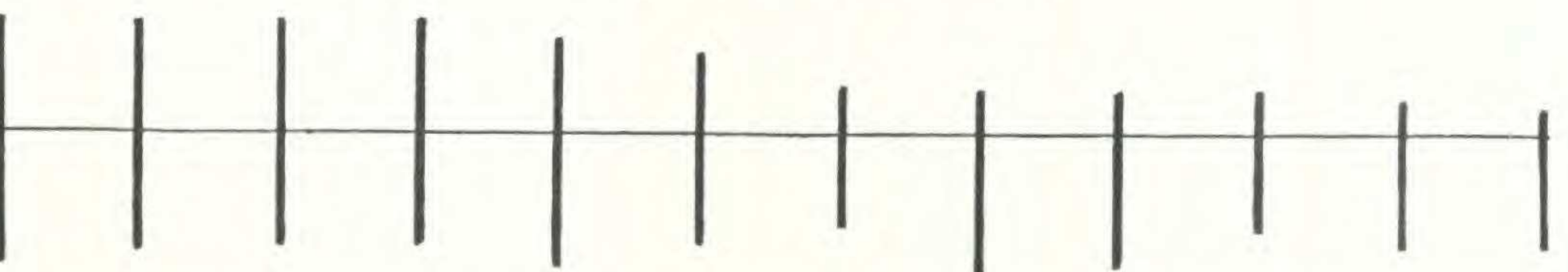


FIGURE 1. DIAGRAMS OF CHROMOSOME COMPLEMENTS. (Explanation in the text.)

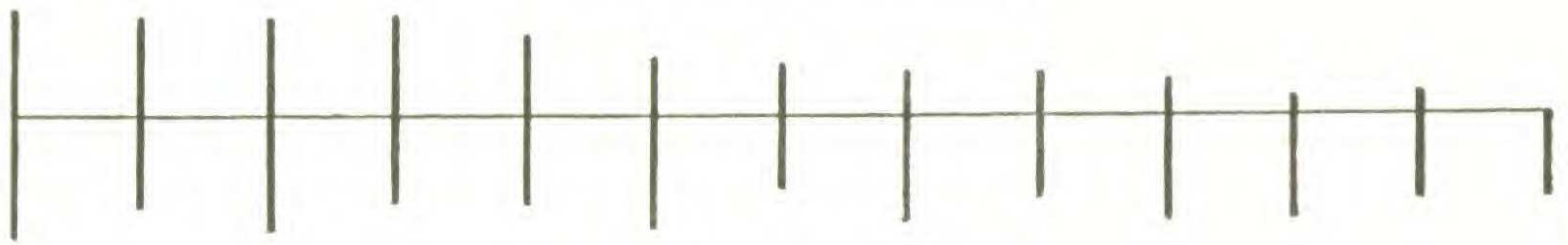
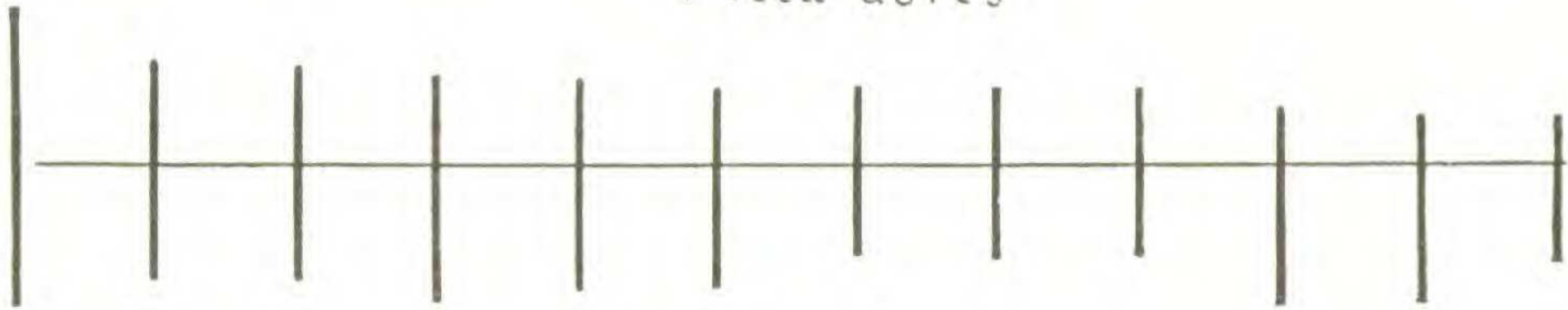
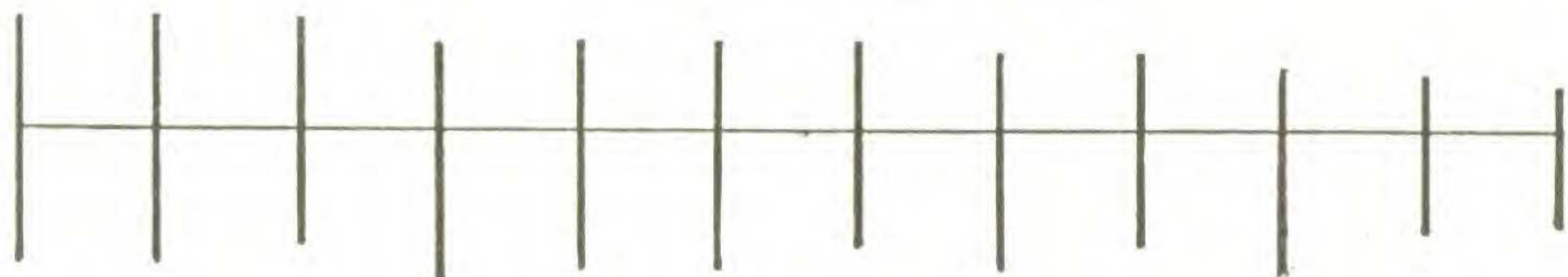
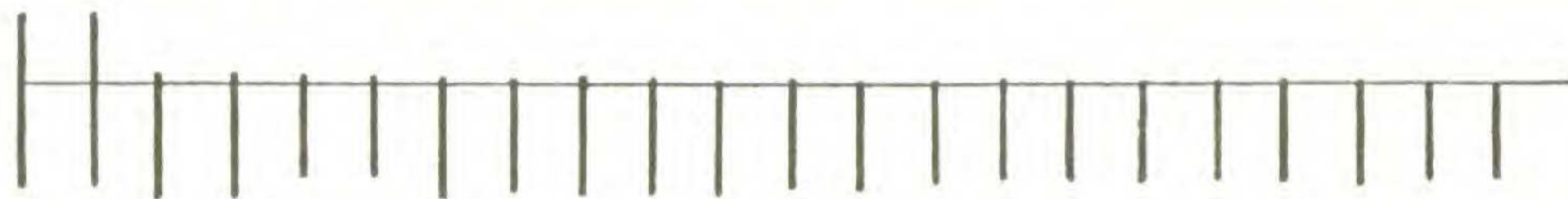
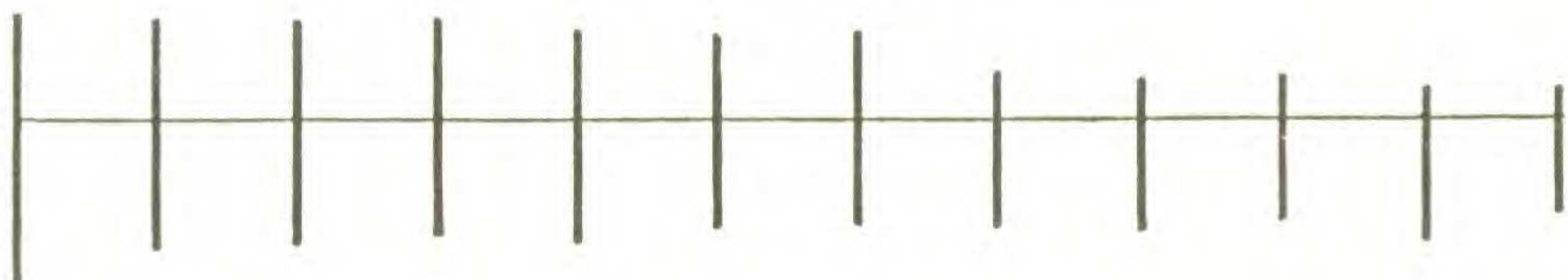
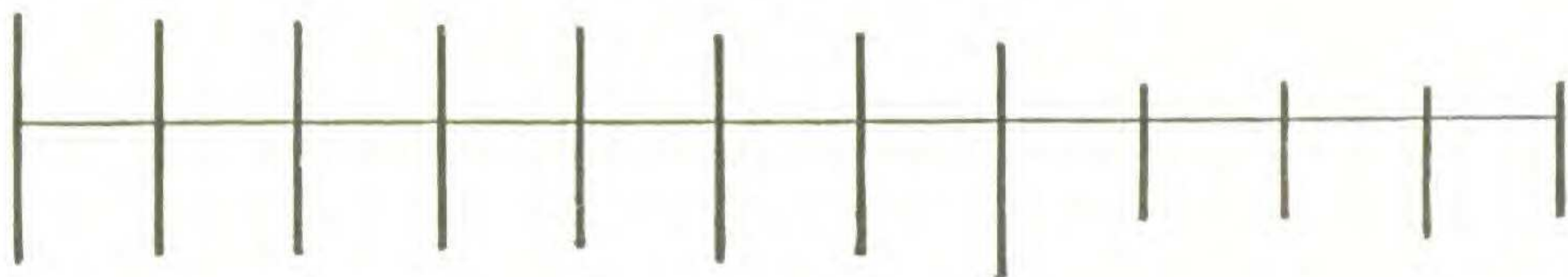
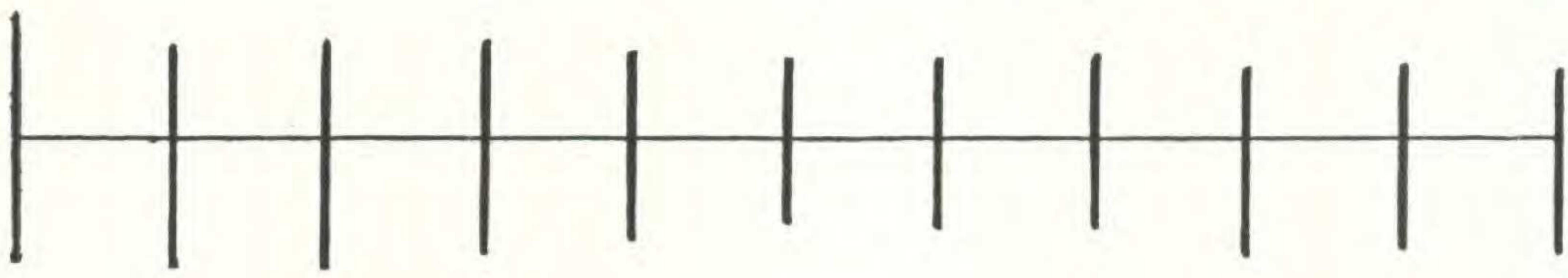
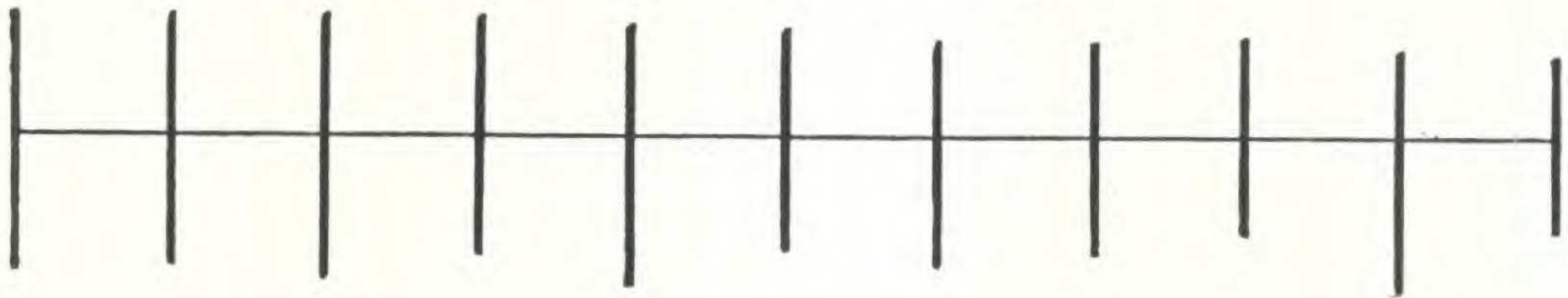
Pseudotsuga taxifolia*Picea abies**Tsuga caroliniana**Pseudolarix amabilis**Abies cephalonica**Abies concolor*

FIGURE 2. DIAGRAMS OF CHROMOSOME COMPLEMENTS. (Explanation in the text.)

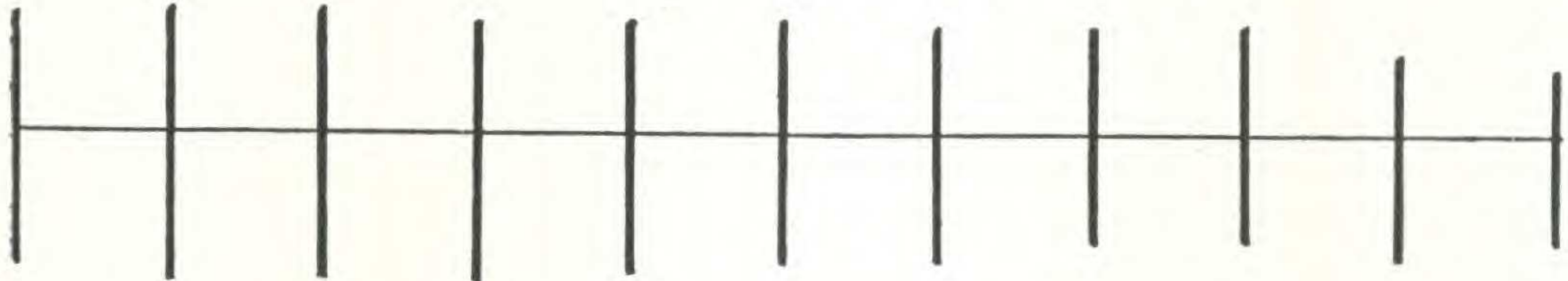
Cryptomeria japonica



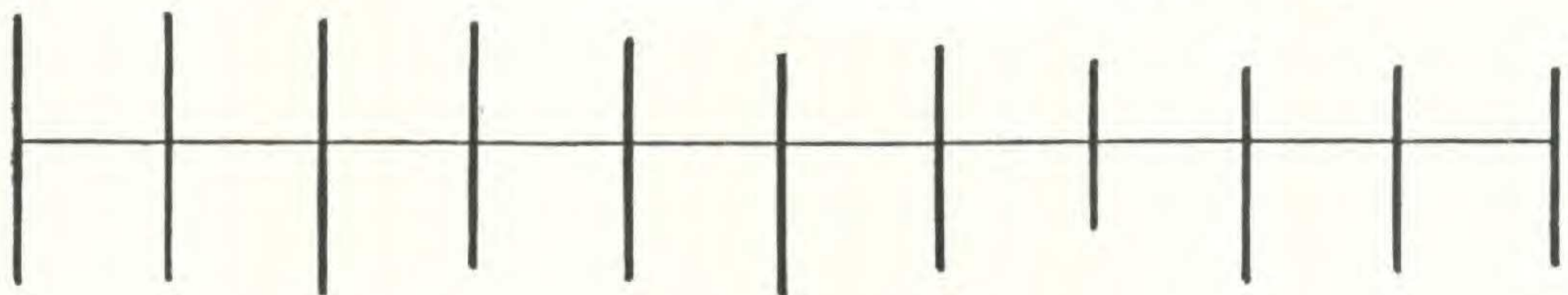
Juniperus virginiana



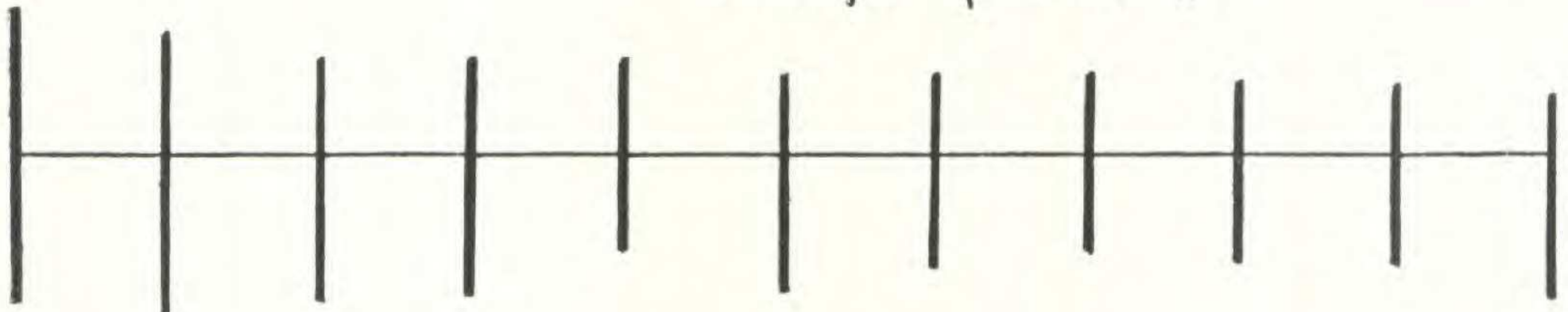
Juniperus chinensis



Chamaecyparis Lawsoniana



Thuja plicata



Thuja orientalis

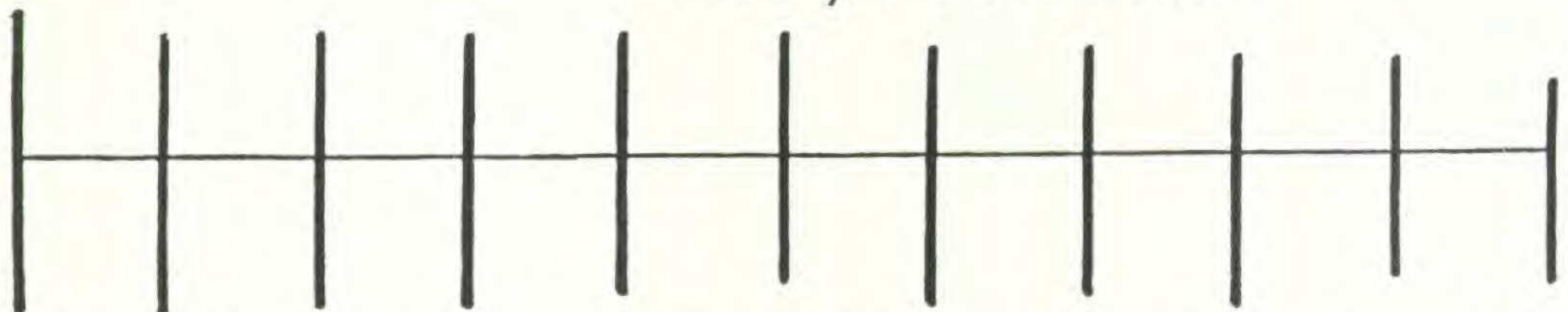


FIGURE 3. DIAGRAMS OF CHROMOSOME COMPLEMENTS. (Explanation in the text.)

types of chiasmata formed. Many of the earlier counts of chromosome numbers in Conifers were only approximations, and relatively little work has been done on the behavior of chromosomes during meiosis.

The material used for this study was obtained from Conifer species grown in the Arnold Arboretum. Chromosome counts were made from meiotic figures in microspore mother cells and divisions in the young microspores. Both aceto-carminic smears, and permanent smears stained with crystal violet, were used.

The reduction divisions in the male flowers occur at different times for different genera and species. In *Juniperus virginiana* and *J. chinensis* the reduction divisions are found in late August; in *Taxus* they occur in October; in *Larix* they are found in late February or early March, although the prophase stages begin in the fall. Reduction divisions occur in the spring in most of the other genera, and in *Juniperus rigida* and *J. communis*. In *Pinus*, few species overlap in time of meiotic divisions, and more than a month elapses between meiosis in *P. Banksiana* and *P. Strobus*. *Cedrus libanotica*, as grown in the Arnold Arboretum, is exceptional in going through meiosis early in August and shedding its pollen in early fall. The female cones develop, and seeds are produced.

The reduction divisions in many species occur almost simultaneously, and not more than two or three days elapse between the first meiotic divisions and the formation of tetrads. All trees of some species seem to undergo reduction divisions about the same time, although there is considerable variation in *Cedrus libanotica*, and in some of species of *Juniperus*.

Both aceto-carminic and permanent smears provided good preparations for a study of chromosome numbers and chiasma frequency. The chiasma frequency does not seem to be very high at mid-diplotene, and most of the chiasmata are not completely terminalized at early metaphase. There are usually somewhat more than two chiasmata per bivalent at metaphase, and these are usually interstitial or sub-terminal. The various stages in meiosis have been photographed to show the typical behavior of the chromosomes in Conifer species. The figures shown in plate 78 are from *Picea Abies*. The double nature of the pachytene "spireme" is not clearly shown in the photograph (Fig. 31), but when flattened, the threads do show the paired chromomeres. The early diplotene stages are not sufficiently distinct for a study of chiasma formation, but at mid-diplotene the chromosomes are clearly defined

in many cases (Fig. 32). The average chiasma frequency at this time is about three per bivalent. At metaphase most of the bivalents are in the form of rings with segments usually projecting beyond the interstitial or subterminal chiasmata. Occasional rod bivalents are found at late metaphase (Fig. 33). As the bivalents divide, their tetrad nature is evident, and the relations of the four chromatids often can be determined in permanent preparations. Frequently two chromatids retain their terminal association after the others have separated (Fig. 34). The chromosomes are easily counted either at metaphase or late anaphase (Fig. 35). A telophase stage of the second division is shown in figure 36. The meiotic divisions appear to be normal, and there is no evidence of chromosome irregularity.

The chiasma frequencies in the chromosomes of different genera of Conifers, and especially in the Abietineae, seem to be very similar. Photographs of meiotic figures of six different genera of Abietineae are shown in plate 79. The meiotic chromosomes of *Larix* have about 2.4 chiasmata per bivalent, as was determined earlier in two species and the F_1 hybrid (Sax, 1932). Most of the chiasmata at diakinesis are interstitial (Pl. 79, fig. 37), and the free distal arms are widely separated. Essentially the same configurations are found in *Pinus* (Pl. 79, fig. 38). There is little terminalization of chiasmata between diakinesis and early metaphase. The first meiotic metaphase in *Tsuga* is shown in Pl. 79, fig. 39. The analysis of chromosome morphology in *Pseudolarix endosperm* showed a total of 22 chromosomes, of which 20 have terminal or subterminal fiber constrictions. The bivalents at meiosis are of the ring-rod type, usually with two chiasmata at the same side of the attachment points (Pl. 79, fig. 40). The meiotic chromosomes of *Cedrus* (Pl. 79, fig. 41) and of *Abies* (Pl. 79, fig. 42) have approximately the same chiasma frequencies as those of *Picea*, *Larix*, and *Pinus*. In general, there is a striking similarity in the meiotic figures of the different diploid Conifers.

A detailed study of chiasma frequency at meiosis has been made for 22 species of Conifers. The only genus represented in the Taxaceae is *Taxus*; but in the Pinaceae two of the sub-families are represented, and all genera of the Abietineae, with the exception of *Keteleeria*, have been examined. The chiasma frequencies are shown in the following table (Table I). Authorities for species names are those given by Rehder (1927).

TABLE I.
CHROMOSOME NUMBER AND CHIASMA FREQUENCY

Species	Chromosome Number	Chiasma frequency					Ave. xta frequency per 5 bivalent
		0	1	2	3	4	
<i>Abies cephalonica</i>	12		8.2	55.1	28.9	7.6	2.4
<i>Abies Nordmanniana</i>	12		6.5	55.9	29.6	8.8	2.4
<i>Cedrus libanotica</i>	12		.8	58.6	29.7		2.5
<i>Juniperus communis</i>	11		7.5	63.3	24.1	5.0	2.2
<i>Larix decidua</i>	12		4.0	63.0	25.0	8.0	2.4
<i>Larix eurolepis</i>	12	0.7	8.0	48.0	32.0	10.7	0.1 2.4
<i>Larix Kaempferi</i>	12		10.0	40.0	25.0	18.0	0.7 2.5
<i>Picea Abies</i>	12		1.7	50.8	30.8	10.0	6.7 2.7
<i>Picea mariana</i>	12		2.7	42.3	43.0	11.1	0.7 2.6
<i>Pinus Banksiana</i>	12		8.3	58.3	26.1	7.2	2.3
<i>Pinus Jeffreyi</i>	12	0.6	7.7	60.1	25.6	5.9	2.4
<i>Pinus nigra</i>	12		6.5	55.5	29.6	8.3	2.4
<i>Pinus Strobus</i>	12		4.2	53.7	29.6	12.5	2.5
<i>Pinus Thunbergiana</i>	12		4.5	61.4	26.5	7.6	2.4
<i>Pseudolarix amabilis</i>	22	0.7	8.7	67.5	19.9	3.1	2.1
<i>Taxus cuspidata</i>	12	0.4	15.1	71.1	12.3	0.9	2.0
<i>Taxus Hunnewelliana</i>	12		15.3	79.5	5.1		1.9
<i>Taxus media</i>	12	5.0	8.8	83.0	7.7		2.0
<i>Thuja Standishii</i>	11		9.2	75.0	15.8		2.1
<i>Tsuga canadense</i>	12		9.1	77.2	12.8	0.7	2.1
<i>Tsuga caroliniana</i>	12	0.6	7.1	57.0	25.6	9.6	2.4
<i>Tsuga diversifolia</i>	12		6.1	70.0	21.6	1.6	2.2

In the above table the proportion of chromosomes with a given chiasma frequency is given in percentage. This was done to facilitate comparisons of chiasma frequencies in different species. With one exception all chiasma frequencies were based on at least 10 pollen mother cells which, in most species, involved 120 or more bivalent chromosomes.

The chiasma frequencies are similar for all genera, ranging from 1.9 to 2.7 chiasmata per bivalent chromosome. The species within each genus have essentially the same chiasma frequencies, and the differences found are probably not significant. The chiasma frequency found in *Taxus* agrees with Dark's (1932) observations.

The meiotic divisions were very regular, and unpaired chromosomes were found only in rare cases. Even the species hybrids show regular divisions and a high percentage of fertile pollen. The pollen sterility was determined for all species used in the analyses of chiasma frequencies and for most of those used for studies on somatic chromosomes. In most cases the pollen sterility ranged from less than 1 percent to 5 percent. The species with more than 5 percent pollen sterility included *Abies cephalonica* (10%), *A. Nordmanniana* (7%), *Juniperus chinensis* (10%), *J. communis* (18%), *Taxus cuspidata nana* (8%), and *T. media* (9%).

There is evidence of well defined polarity in the development of the microspores of Conifers. In the winged pollen grains, the first division in the microspore is at right angles to the axis of the wings and near the heavy wall of the cell. The nucleus nearer the wall disintegrates and the other divides. Again the nucleus near the wall disintegrates, and the remaining nucleus divides to form the tube and generative nuclei. The nucleus nearer the cell wall becomes the generative nucleus which ultimately produces the male gametes. In rare cases the divisions are oriented parallel to the axis of the wings. In such cases four nuclei of equal size are produced, and there is no evidence of any differentiation. Apparently nuclear differentiation in these microspores is dependent on polarity.

Chromosome numbers were also determined in a number of species where adequate material was not available for a study of chiasma frequencies. The number of bivalents found at meiosis was 12 in *Abies Veitchii*, *Larix occidentalis*, *Picea glauca*, *Pinus echinata*, *P. ponderosa*, *P. pungens*, *P. rigida*, *P. sylvestris*, *P. tabulaeformis*, *P. virginiana* and *Taxus canadensis*. *Juniperus chinensis Pfitzeriana* is undoubtedly a tetraploid with about 22 pairs of chromosomes. All other species of *Juniperus* studied have 11 pairs of chromosomes. *Cryptomeria japonica* also has 11 pairs of chromosomes at meiosis.

DIFFERENTIATION OF SPECIES AND GENERA OF CONIFERS

The basic chromosome number seems to be 12 for most Conifers and other typical Gymnosperms. The chromosome counts listed by Tischler (1927, 1931) include 8 genera of Cycadaceae, all of which have 12 chromosomes, with one doubtful exception. Several investigators have found 12 pairs of chromosomes in *Ginkgo*. Less than 12 chromosomes have been found in *Taxus* and *Torreya* by earlier workers, but Ishikawa's count of 12 in *Taxus* has been confirmed by the

work of Dark and by our studies. Burlingame, Ishikawa, and Schurhoff have found 12 chromosomes in several species of *Podocarpus*. Twelve is the basic number reported in *Larix*, *Pinus*, *Picea*, *Tsuga*, and *Abies*. The earlier counts in the Araucarineae, Cupressineae, and Taxodineae were obtained in connection with morphological studies, and for the most part they are of doubtful value. The Gnetales are exceptional types of Gymnosperms and seem to be exceptional in chromosome number. The most reliable counts in this family seem to be those of Geitler, who finds 7 pairs of chromosomes in *Ephedra*. Twelve appears to be the original basic number for all other Gymnosperms.

Deviations from the typical basic chromosome number are found in the Conifers, but they can be attributed to the addition or loss of a chromosome, or to polyploidy. Thirteen chromosomes are found in *Pseudotsuga taxifolia* endosperm cells. Meiotic divisions were not observed in this genus, so that it is not possible to say whether the extra chromosome is a simple duplication, or a duplication followed by interchange of segments and loss of complete homology with any one of the twelve chromosomes of the basic complement.

Cryptomeria, *Thuja*, *Juniperus*, and *Chamaecyparis* each have 11 chromosomes as a basic number. If 12 is the basic chromosome number in the Conifers, it is improbable that the lower number is the result of a loss of an entire chromosome. The loss of a single chromosome from the basic complement produces inviable gametes and zygotes in practically all plants. It seems more probable that interchange of segments left one chromosome too short to pair regularly. If such a chromosome carried only unessential genes, it could be eliminated with no lethal effect on either gametes or zygotes.

Pseudolarix has two isobrachial chromosomes and twenty with terminal or subterminal fiber attachment points. The chromosome morphology of this species might suggest that the original chromosome complement consisted of twelve more or less isobrachial chromosomes, of which ten had segmented at or near the fiber attachment. This suggestion is in accord with the fact that there are few multivalent associations of chromosomes at meiosis. An increase in chromosome number by fragmentation would also necessitate a corresponding increase in fiber attachment points, and there is no evidence that such attachment points can be divided or formed *de novo*. To be sure Darlington (1929) has postulated that chromosome fragments of a certain size may form new fiber attachments, but as Navashin (1932) has pointed out, the evidence against this hypothesis is very conclusive.

Chromosome fragments of any size do not survive unless they retain the fiber attachment or are attached to another chromosome possessing it. It is improbable, therefore, that the chromosome number of *Pseudolarix* can be attributed to fragmentation. If *Pseudolarix* is a polyploid with twelve chromosomes as the original basic number, the aneuploid condition caused by the loss of two chromosomes would not necessarily be deleterious, especially if preceded by segmental interchange. Interchange between many of the remaining non-homologous chromosomes might reduce homology to such an extent that few multivalent associations would be found at meiosis, even though the chiasma frequency exceeds two per bivalent. Homology of several chromosomes in the haploid complement is shown in dividing figures in endosperm tissue. In one metaphase figure two chromosomes were in contact at all loci, and several other chromosomes were found closely associated in pairs.

Polyploidy is rare in the Conifers and has been reported previously only in *Sequoia sempervirens*. Dark (1932) found about 50 chromosomes in the root tips of this species and considers it to be a tetraploid. Our aceto-carminic preparations of root tips also show that this species is a polyploid. Although the exact number of chromosomes could not be determined, there were more than 40. The somatic number in *Sequoia gigantea* is from 21 to 24 chromosomes, according to Goodspeed and Crane (1920). *Juniperus chinensis Pfitzeriana* is undoubtedly a tetraploid with 22 pairs of chromosomes. The other Juniper species examined have 11 pairs of chromosomes. The three polyploid species of Conifers described are presumably auto-polyploids, although changes in chromosome morphology may have reduced homology subsequent to chromosome duplication.

It is evident that changes in chromosome number have played a minor part in the differentiation of genera of Conifers. The Cupressineae differ from most other Conifers in having a basic chromosome number of 11. The closely related sub-family Taxodineae may also have 11 chromosomes as the basic number. According to Dark (1932), *Cryptomeria* has 24 somatic chromosomes, but counts from root tips are unsatisfactory and may not be reliable. We have found 11 chromosomes in the endosperm cells of *Cryptomeria* and in meiotic figures. Coker (1903) pictures 11 chromosomes in the microspore division in *Taxodium*. Goodspeed and Crane (1920) found 21 to 24 chromosomes in root tips of *Sequoia gigantea*, which might be taken to mean that the somatic number was less than 24. We have also found about 22 somatic chromosomes in *Taiwania* and *Taxodium*.

Differentiation of genera of Conifers seems to be associated, to a considerable extent, with differences in chromosome morphology. The two genera of Taxaceae studied are somewhat similar in chromosome morphology, although two of the chromosomes of *Taxus* are more nearly subterminal than those of *Cephalotaxus*. The Abietineae show considerable variation. All chromosomes of *Pinus* are approximately isobrachial; one chromosome of *Cedrus* is distinctly heterobrachial; six chromosomes of *Larix* are heterobrachial; and all but two of the twenty-two chromosomes of *Pseudolarix* have terminal or subterminal fiber attachments. *Picea*, *Abies* and *Tsuga* show some variation in length of chromosomes and positions of fiber attachments. In the Cupressineae, however, there is little difference in chromosome morphology of different genera. The chromosomes of *Juniperus*, *Thuja*, and *Chamaecyparis* are similar in morphology and are all more or less isobrachial. The chromosomes of *Cryptomeria*, representing the Taxodineae, resemble those of the Cupressineae. These two sub-families seem to be similar in chromosome number and chromosome morphology.

Where different genera show well marked differences in chromosome morphology, one might suppose that such variation is responsible for the initial isolation which would permit independent variation and the ultimate differentiation of genera. If changes in chromosome morphology are caused primarily by segmental interchange, as seems probable, then the new interchange types may be isolated from the original type because of the sterility of individuals heterozygous for segmental interchange. A high degree of sterility in individuals heterozygous for segmental interchange chromosomes would be expected in the Conifers because of the prevalence of interstitial chiasmata at meiosis. The rigidity of interchange rings caused by interstitial chiasmata would result in a high percentage of non-disjunction and consequently a high gametic sterility (Sax and Anderson, 1933). Only the forms homozygous for segmental interchange could survive, and variations produced in these forms would not be swamped by intercrossing with the original parental types with a different chromosome morphology. A considerable amount of interchange and natural selection would have to occur to produce the variations found in certain genera.

Differentiation of genera, and even sub-families, in the Cupressineae and Taxodineae has occurred with little apparent change in chromosome morphology. Exchange of approximately equal segments could occur with no apparent change in chromosome morphology, as is the case in *Tradescantia* (Sax and Anderson, 1933). On the other hand,

it is quite possible that the differentiation of these genera may be initiated and maintained by genetic factors, as seems to be the case in certain races and species of *Drosophila* (Schultz and Dobzhansky, 1933; and Dobzhansky, 1933).

The factors involving the differentiation of genera in the Conifers seem to be of a different nature from those responsible for species differentiation. In the Abietineae, especially, the genera differ considerably in chromosome morphology, but the species within a genus show little or no variation in chromosome morphology. If species differentiation in the Conifers is caused primarily by gene mutations, many species within a genus may not differ sufficiently to prevent species hybridization and fertility of the F_1 hybrids. The available evidence seems to support this assumption. *Larix eurolepis* is an F_1 hybrid between *L. Kaempferi*, a native of Japan, and the European Larch, *L. decidua*. Although the parental species have been isolated for a long time, the F_1 hybrid shows almost complete chromosome pairing, the chiasma frequency at meiosis is as high as that found in the parents, and more than 90 percent of the pollen is good (Sax, 1932). According to Rehder (1927), *Taxus media* is a hybrid of the Japanese Yew, *T. cuspidata*, and the English Yew, *T. baccata*. *Taxus Hunnewelliana* is supposed to be a hybrid between *T. cuspidata* and *T. canadensis*. Both of these hybrids show regular chromosome pairing, normal chiasma frequency at meiosis, and a high degree of fertility. Species hybrids have been found in *Abies*, *Tsuga*, *Picea*, *Larix*, and *Pinus* (Rehder, 1927). According to Austin (1927), there are seven known natural species hybrids in *Pinus* and two of artificial origin. Austin has made additional crosses which seem to be successful.

If different species cross readily, there must be some factors which prevent extensive crossing and thus maintain the species as units. Two factors certainly play a part in species isolation,—geographic distribution and time of flowering. Although the ranges of certain species overlap, many species are so separated in distribution that crossing could never occur in nature. The shedding of pollen in most species is completed in a few days, and there is often no overlapping of flowering periods within species of a given genus. For example, *Tsuga canadensis* sheds its pollen before *T. caroliniana* or *T. diversifolia*. The last two species flower at the same time in the Arboretum, but in nature they are geographically isolated. Artificial crosses have been made between these two species which have resulted in an abundant set of seed. The species of *Pinus* also show a great range in time of flower-

ing, and more than a month elapses between the pollination of *P. Banksiana* and pollination of *P. Strobus*. Many species of *Pinus* are undoubtedly interfertile, but certain species in different sections may be so genetically differentiated that the artificial production of hybrids would be difficult or impossible.

The chromosome numbers in the Gymnosperms are of interest in regard to the absence of double fertilization in this group of plants. According to Müntzing (1933), hybrid incompatibility and the occurrence of polyploidy in the Angiosperms are more or less dependent on double fertilization. Polyploidy is preserved because of incompatibility between diploids and polyploids, caused by the disturbed relations between embryo, endosperm, and somatic tissues. As evidence in support of this view, Müntzing points out the rare cases of polyploidy in genera which do not have double fertilization.

Although polyploidy is rare in the Conifers, at least two polyploid species are known, *Juniperus chinensis Pfitzeriana* and *Sequoia sempervirens*, and it is probable that *Pseudolarix*, also, is of polyploid origin. One might suppose that the prevalence of interstitial chiasmata in the Conifers would result in quadrivalent chromosome configurations at meiosis of such a nature that irregular chromosome distribution would occur and produce considerable pollen sterility, but the pollen sterility in the tetraploid Juniper is only 6 percent, which is less than that found in some of the diploid species. There is less than 5 percent of pollen sterility in *Pseudolarix*. It is evident that the rare occurrence of polyploidy in the Conifers can not be attributed to the sterility of such types.

According to Müntzing's theory polyploid Conifers are rare because diploid and polyploid forms can cross readily, and consequently the polyploid form is not isolated and can not develop independently unless geographically isolated. This hypothesis might also explain the differentiation of relatively few species and genera in the Gymnosperms.

SUMMARY

The authors have determined the chromosome numbers for 53 species representing 16 genera of Conifers. The basic chromosome number is 12 for most Gymnosperms, with the exception of the Gnetales. Twelve pairs of chromosomes have been found in all genera studied in the Taxaceae. Deviations from the typical basic number are found in the Pinaceae. In the Abietineae 12 pairs of chromosomes are found in *Picea*, *Tsuga*, *Abies*, *Larix*, and *Pinus*, as reported by earlier investi-

gators. *Cedrus* also has 12 chromosomes, but in *Pseudotsuga* there are 13, and in *Pseudolarix* there are 22 pairs of chromosomes. The basic number is 11 in the Cupressineae as represented by *Thuja*, *Juniperus*, and *Chamaecyparis*. One variety of *Juniperus chinensis* is a tetraploid. The basic number seems to be 11 in the Taxodineae examined also. *Cryptomeria* undoubtedly has 11 pairs of chromosomes, and the same number was found in *Taxodium* and *Taiwania*. *Sequoia sempervirens* is a polyploid, but the exact number of chromosomes could not be determined. Deviations from the primary basic number are attributed to the loss of a small chromosome, following translocation of segments, in the Cupressineae and Taxodineae; duplication of a chromosome in *Pseudotsuga*; and polyploidy in three species of Conifers.

Genera of Conifers may differ considerably in chromosome morphology, but species within each genus are very similar. Differentiation of genera is often associated with changes in chromosome morphology, presumably caused by segmental interchange. Species differences seem to be based primarily on genic changes, which, in many cases, do not prevent compatible species hybrids. Many species maintain themselves as distinct units only by geographic or physiological isolation.

The chiasma frequency and behavior of the chromosomes at meiosis has been determined for 22 species representing 10 genera of Conifers. The average number of chiasmata per bivalent ranges from about 2.0 to 2.7. The meiotic figures are somewhat similar in all diploid species examined, and especially so in the Abietineae. The similarity in chromosome numbers and meiotic configurations in the Conifers is remarkable as contrasted with the great variation in numbers and chiasma frequencies found in the Angiosperms.

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EXPLANATION OF THE PLATES

Plate 75.

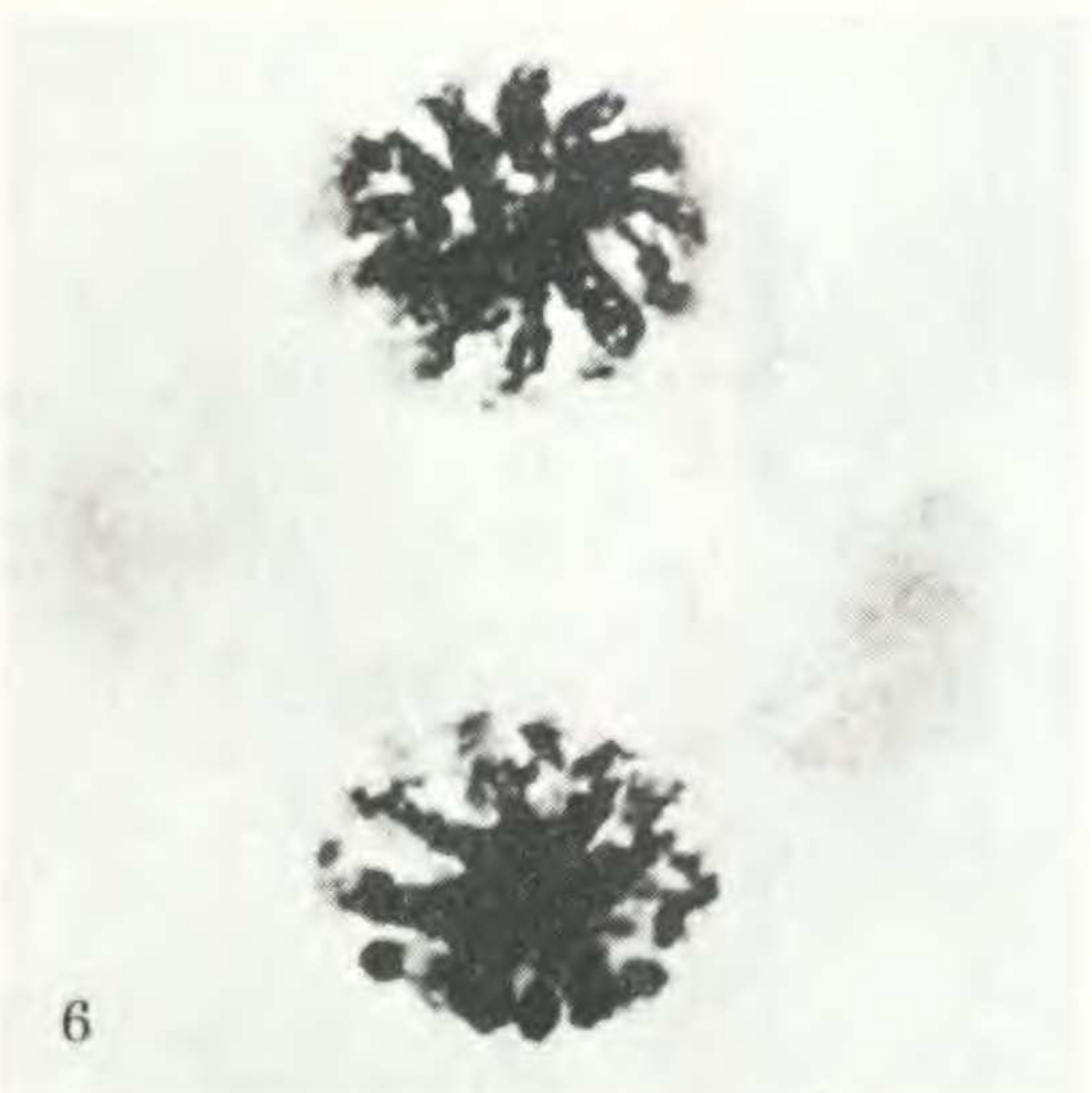
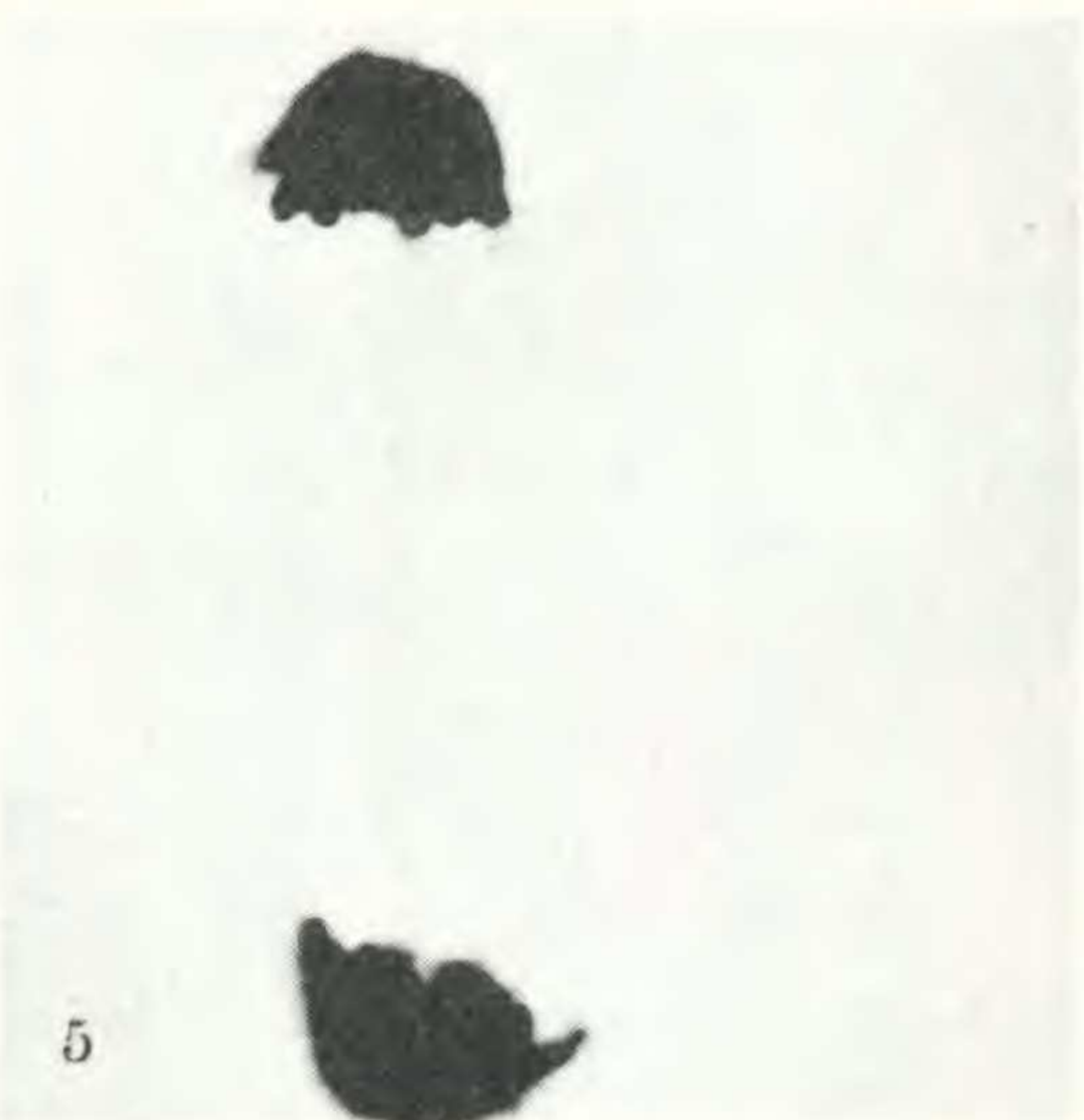
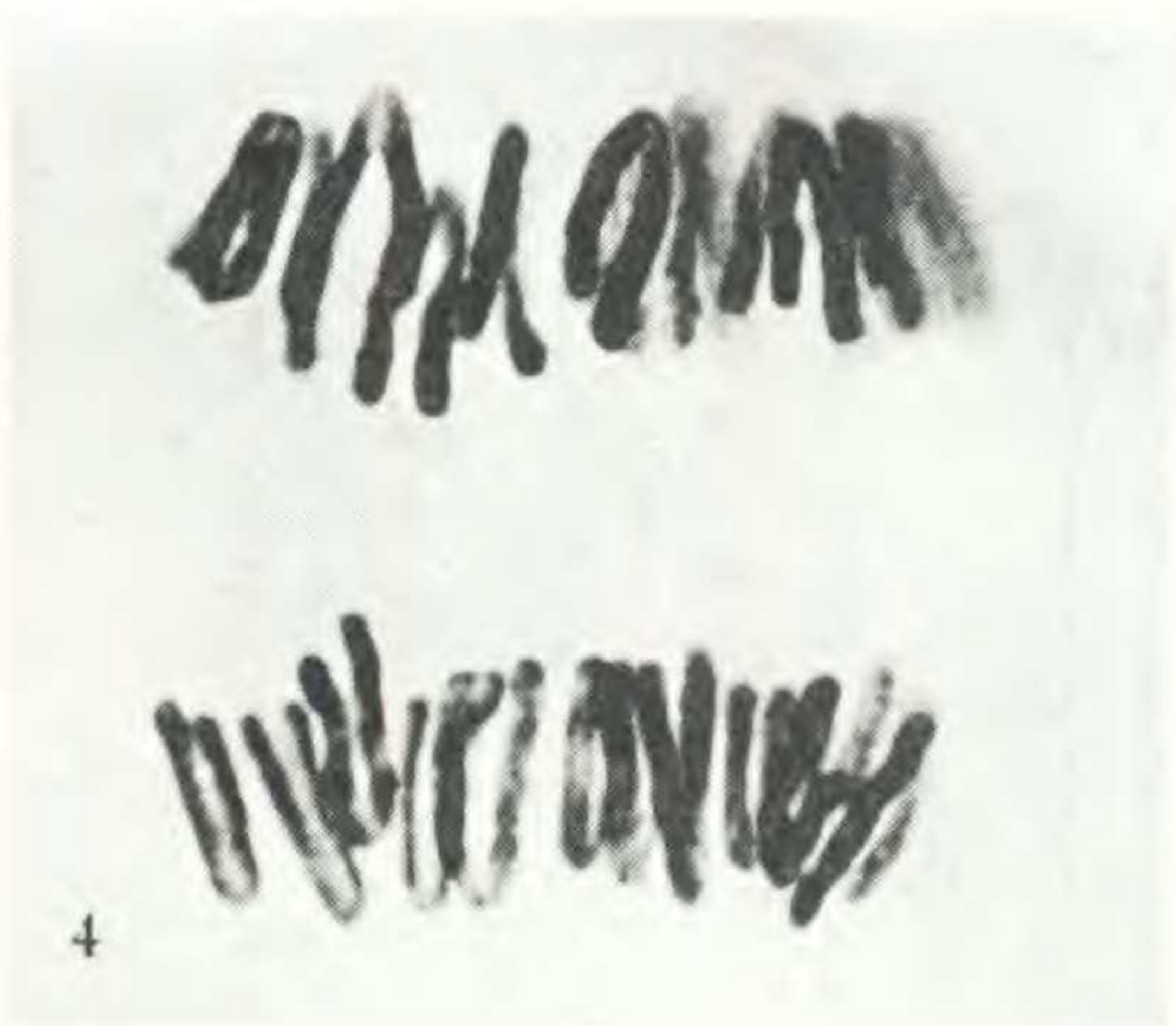
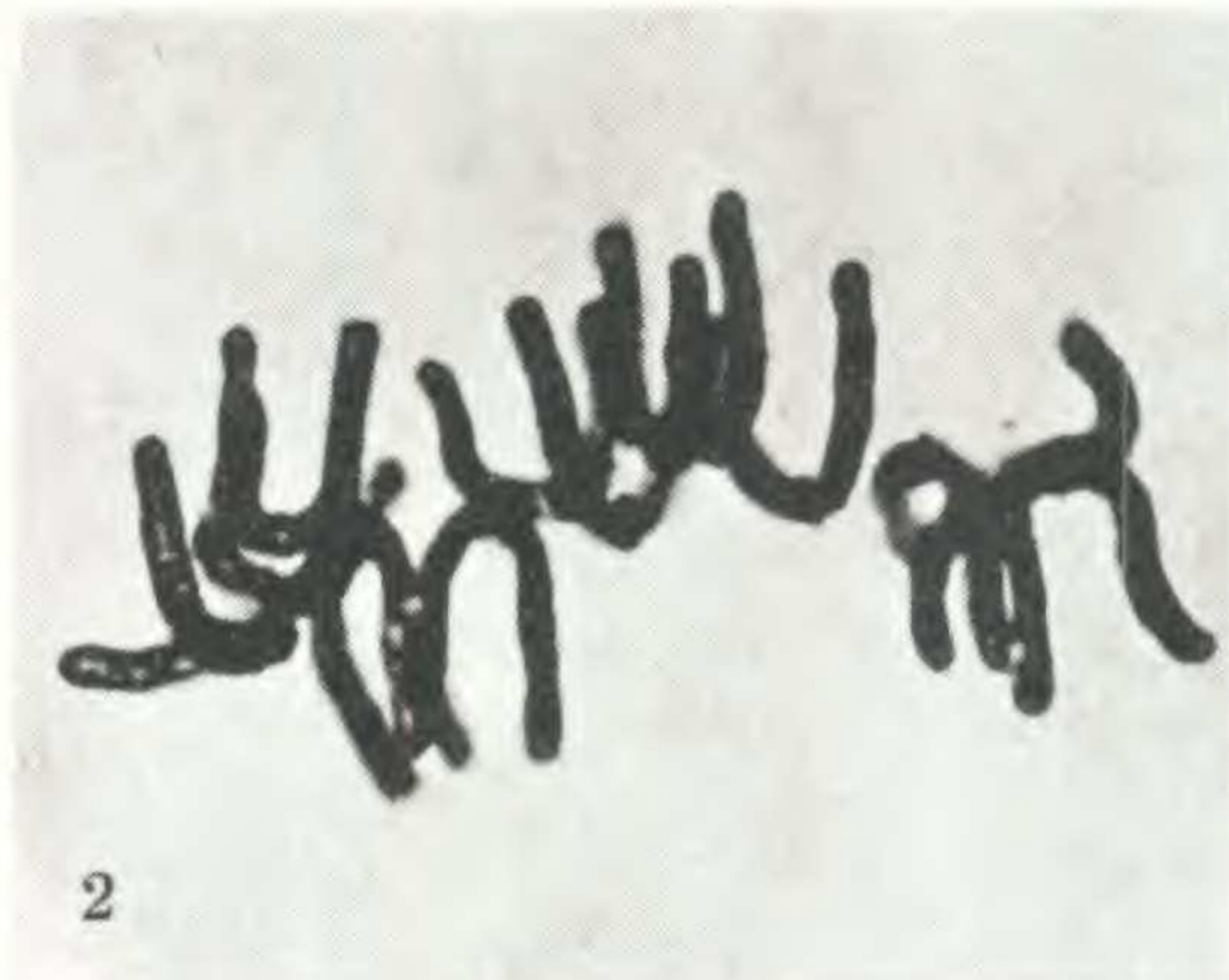
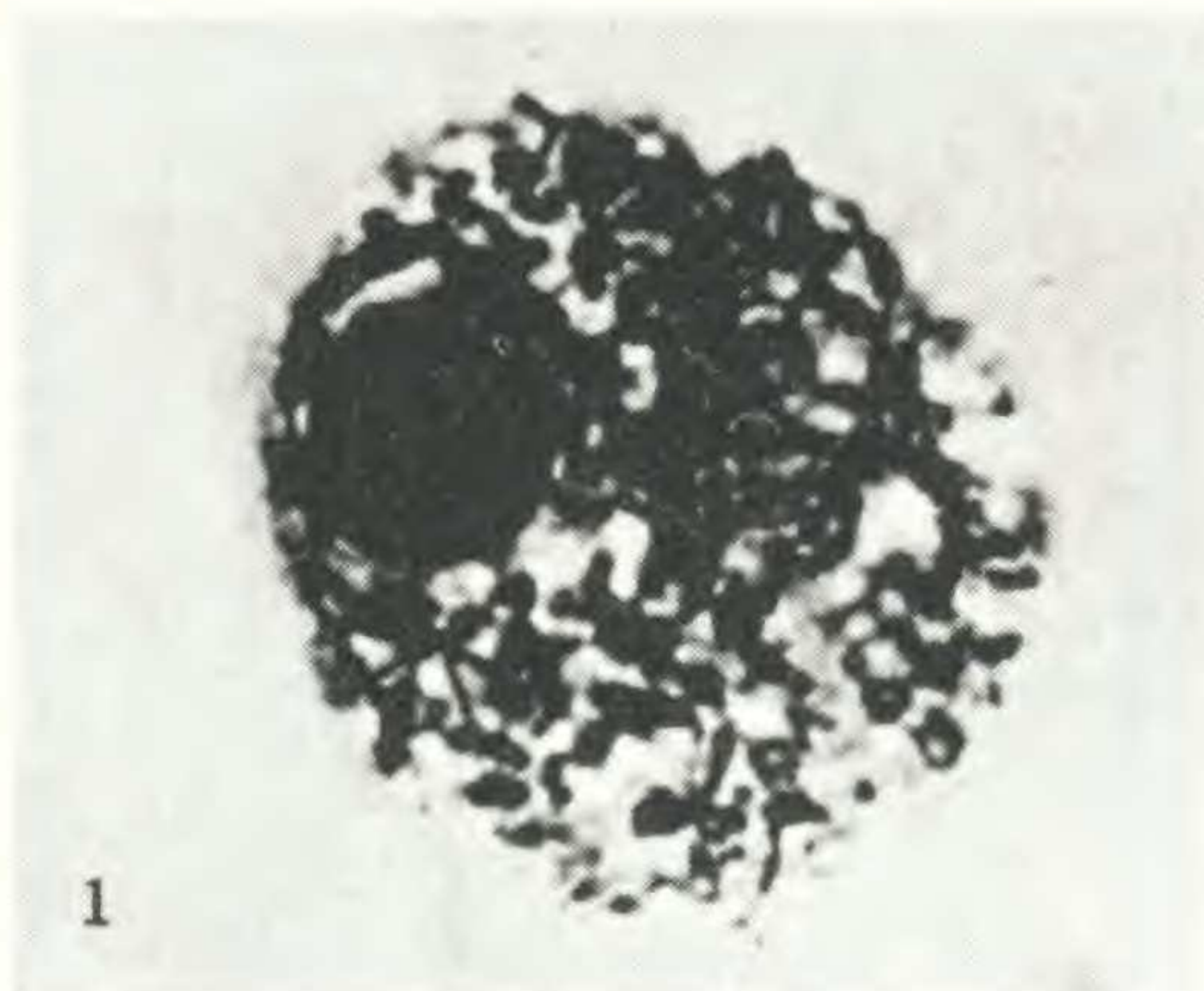
Photographs of different stages in the mitotic cycle in the endosperm of *Cephalotaxus Fortunei*.

- Fig. 1. Prophase showing "spireme" and single nucleolus.
- Fig. 2. Metaphase. The fiber attachment points can be seen in several chromosomes.
- Fig. 3. Anaphase. Separation of daughter chromosomes almost complete.
- Fig. 4. Late anaphase. The number and types of chromosomes are easily determined.
- Fig. 5. Late telophase. The chromosomes form a compact mass at each pole.
- Fig. 6. Reconstruction of daughter nuclei. The chromosomes are polarized around a single nucleolus.

Plate 76.

Somatic chromosomes from endosperm tissue of Conifers. Drawn from aceto-carmin preparations, $\times 1200$.

- Fig. 7. *Ginkgo biloba*. Metaphase. 12 chromosomes.
- Fig. 8. *Taxus canadensis*. Metaphase. 12 chromosomes.
- Fig. 9. *Taxus baccata*. Metaphase. 12 chromosomes.
- Fig. 10. *Taxus cuspidata*. Metaphase. 12 chromosomes.
- Fig. 11. *Pinus parviflora*. Metaphase. 12 chromosomes.
- Fig. 12. *Pinus Thunbergiana*. Metaphase. 12 chromosomes.
- Fig. 13. *Pinus ponderosa*. Metaphase. 12 chromosomes.
- Fig. 14. *Cedrus libanotica*. Metaphase. 12 chromosomes.
- Fig. 15. *Larix Kaempferi*. Late anaphase. 12 chromosomes.
- Fig. 16. *Larix decidua*. Metaphase. 12 chromosomes.
- Fig. 17. *Pseudolarix amabilis*. Metaphase. 22 chromosomes.
- Fig. 18. *Picea pungens*. Metaphase. 12 chromosomes.



Conifer Chromosomes